<Division of Environmental Chemistry>Molecular Microbial Science

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– Molecular Microbial Science –

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Scope of Research

Microorganisms are found almost everywhere on Earth. They have a great diversity of capacities to adapt to various environments, including chemically and physically unusual environments. Our main subject is to clarify the molecular basis of environmental adaptations of microorganisms and their application. Specific functions of proteins and lipids with essential roles in environmental adaptation of extremophilic microorganisms are of our particular interest. We also undertake mechanistic analysis of microbial enzymes, in particular, those involved in unique metabolic pathways, and their application.

KEYWORDS

Extremophiles
Bacterial Cold-adaptaion Mechanism
Polyunsaturated Fatty Acid
Phospholipid Acyltransferase
Extracellular Membrane Vesicle

Selected Publications

Kawai, S.; Kawamoto, J.; Ogawa, T.; Kurihara, T., Development of a Regulatable Low-temperature Protein Expression System Using the Psychrotrophic Bacterium, *Shewanella livingstonensis* Ac10, as the Host, *Biosci. Biotechnol. Biochem.*, **83,** 2153-2162 (2019).

Casillo, A.; Di Guida, R.; Carillo, S.; Chen, C.; Kamasaka, K.; Kawamoto, J.; Kurihara, T.; Corsaro, M. M., Structural Elucidation of a Novel Lipooligosaccharide from the Cold-Adapted Bacterium OMVs Producer *Shewanella* sp. HM13, *Mar. Drugs*, **17,** E34 (2019).

Ogawa, T.; Tanaka, A.; Kawamoto, J.; Kurihara, T., Purification and Characterization of 1-Acyl-*sn*-glycerol-3-phosphate Acyltransferase with a Substrate Preference for Polyunsaturated Fatty Acyl Donors from the Eicosapentaenoic Acid-producing Bacterium *Shewanella livingstonensis* Ac10, *J. Biochem*., **164,** 33-39 (2018).

Toyotake, Y.; Cho, H. N.; Kawamoto, J.; Kurihara, T., A Novel 1-Acyl-*sn*-glycerol-3-phosphate *O*-Acyltransferase Homolog for the Synthesis of Membrane Phospholipids with a Branched-chain Fatty Acyl Group in *Shewanella livingstonensis* Ac10, *Biochem. Biophys. Res. Commun.*, **500,** 704-709 (2018).

Tokunaga, T.; Watanabe, B.; Sato, S.; Kawamoto, J.; Kurihara, T., Synthesis and Functional Assessment of a Novel Fatty Acid Probe, ω-Ethynyl Eicosapentaenoic Acid Analog, to Analyze the in Vivo Behavior of Eicosapentaenoic Acid, *Bioconjugate Chem.*, **28,** 2077-2085 (2017).

Bacterial Regulation of Vesicle Production and Biofilm Dispersion in Response to Extracellular Environment

Extracellular membrane vesicles (EMVs) secreted by many kinds of bacteria have various roles in survival such as inter-cellular communication and biofilm formation. Therefore, the amounts and components of EMVs should be tuned in response to their growing environment. Although several vesiculation mechanisms are suggested, it remains largely unknown how bacteria regulate vesiculation in response to the environments. We are focusing on a sensor protein, HM1275, identified in EMVs of *Shewanella vesiculosa* HM13, a cold-adapted Gram-negative bacterium.

Addition of Lys to a poor nutrient medium increased the vesicle production by the parent strain in a dose-dependent manner, whereas the effect of Lys addition on the *hm1275*-disrupted mutant was less significant. HM1275 has approximately 40% sequence identity to BdlA, which is known as a protein for biofilm dispersion. The amount of biofilm of the parent decreased over time probably due to biofilm dispersion and was lower than that of the mutant in the poor medium containing additional Lys (Figure 1).

Together, HM1275 is involved in regulation of both vesicle production and biofilm dispersion in response to Lys in the poor nutrient medium. There may be a linkage between these two phenomena, where HM1275-containing EMVs released by the Lys-sensing cells are delivered to other cells to induce biofilm dispersion for collective cell behavior.

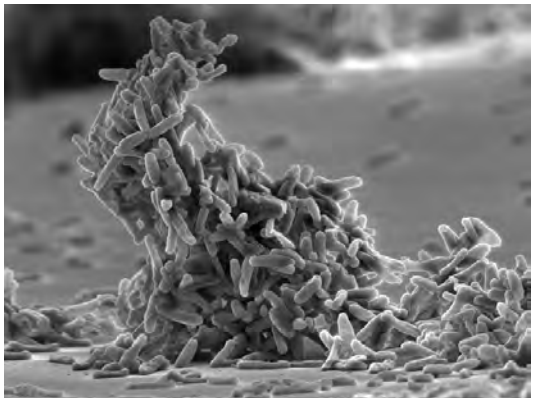

Figure 1. Side-view of SEM image of Δ*hm1275* biofilm.

Bioconversion of DHA into EPA

The psychrotrophic bacterium *Shewanella livingstonensis* Ac10 produces eicosapentaenoic acid (EPA) at cold temperatures. The EPA-less mutant that is deficient of *de novo* EPA biosynthesis showed cold-sensitive phenotypes such as growth retardation and abnormal cell filamentation, and these phenotypes were suppressed by supplementation of EPA and of docosahexaenoic acid (DHA). The interesting finding was that the EPA-less mutant produced EPA when grown in the presence of DHA, suggesting the presence of the unidentified metabolic pathway that converts DHA into EPA (Figure 2A). The biosynthesis of EPA/DHA in marine bacteria has been intensively studied, whereas the other metabolic route including degradation and bioconversion are poorly understood. To understand the DHA conversion mechanism in *S. livingstonensis* Ac10, we carried out mutagenesis experiments of genes of β-oxidation enzymes and their homologs. We found that the disruption of *sl_1351* gene that putatively encodes 2,4-dienoyl-CoA reductase (FadH) resulted in the decreased conversion level (Figure 2B). FadH is an auxiliary enzyme of β-oxidation pathway and essential to degrade Δ^4-unsaturated fatty acids, like DHA. As Sl_1351 is highly homologous to a well-studied FadH from *Escherichia coli*, the DHA conversion is likely mediated through a typical β-oxidation pathway. On the other hand, possible β-oxidation intermediates other than EPA (*e.g.* octadecapentaenoic and hexadecatetraenoic acids) were not detected on the supplementation of DHA. It suggests that *S. livingstonensis* Ac10 metabolizes DHA to preferentially form EPA, which is an important biofactor for the bacterium.

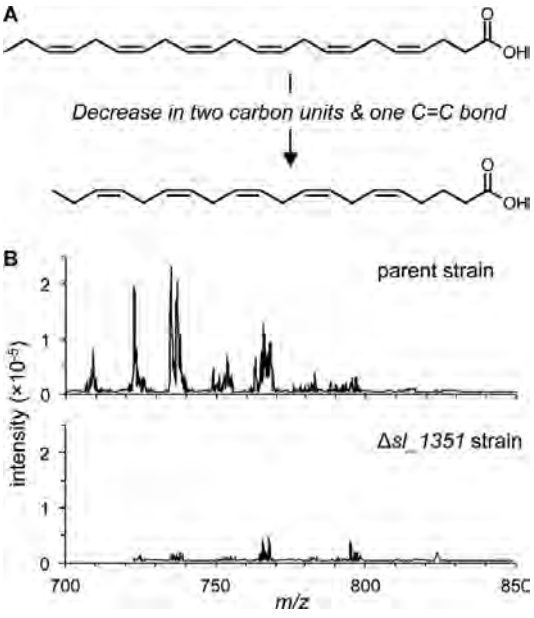

Figure 2. (A) The schematic view of the bioconversion from DHA to EPA. (B) Mass spectrometry analysis of EPA-containing phospholipids from the parent and Δ*sl_1351* cells. The precursor ion scans for the target ion of *m/z* 301 (corresponding to $[M\text{-}H]^-$ ion for EPA) are shown.